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The four-plates test: Anxiolytic or analgesic paradigm?

Nadège Ripoll, Martine Hascoët, Michel Bourin *

EA 3256, Neurobiologie de l'anxiété et de la dépression, Faculté de Médecine, BP 53508, 1 rue Gaston Veil, F44035 Nantes cedex 01, France

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Abstract

The four-plates test (FPT) is an animal model of anxiety in which the exploration of the novel surroundings is suppressed by the delivery of a mild electric foot shock. The anti-nociceptive system has been reported to be activated by a variety of stressful stimuli such as footshock. The present study was thus designed to compare effects of drugs in the FPT and in the hot-plate test (an animal model of pain), in order to disambiguate the drug-induced anti-punishment effects obtained in the FPT from alterations in pain sensitivity. Various compounds, known to be implicated in anxiety states as well as nociception, have been studied. Although morphine induced a strong anti-nociceptive effect, it did not modify the number of shocks received in the FPT. Alprazolam and diazepam induced an anxiolytic-like effect in the FPT, at doses that did not induce any effect in the hot-plate test. The antidepressants previously reported anxiogenic (desipramine, maprotiline) in the FPT were found to be analgesic at the same doses. Milnacipran, venlafaxine and paroxetine did not modify the pain threshold, whereas they have previously been shown to induce anxiolytic-like effects in the FPT. The dopaminergic antidepressant agent nomifensine was without effect on both tests. Our results suggest that the reported drug-induced anti-punishment effects in the FPT are not related to modifications of pain threshold but to a pure anxiolytic-like effect.

Keywords: Antidepressants; Aversive states; Benzodiazepines; Four-plate test; Hot-plate test; Nociception

1. Introduction

The four-plates test (FPT) is an animal model of anxiety based on spontaneous response (Aron et al., 1971). Animals are exposed to a novel environment. The exploration of this novel surrounding is suppressed by the delivery of mild electric foot shock contingent to quadrant crossing. Animal can only escape from this aversive situation by remaining motionless (passive avoidance). This model of conditioned fear presents several advantages. It is a simple and quick procedure and there is no need for prior training of animals. In this test, benzodiazepines (BZDs) induce a strong anti-punishment effect, which has been proposed to be a reflection of their anxiolytic activity (Bourin et al., 1992). The FPT also allows the detection of anxiolytic effects of other non-BZD anxiolytic compounds such as selective serotonin (5-HT) reuptake inhibitors (SSRI) or mixed serotonin and noradrenaline (NA) reuptake inhibitors (SNRI) (Hascoët et al., 2000). Substance P and opioid systems as well as other systems, such as serotonergic, noradrenergic, GABAergic and dopaminergic systems, are implicated in nociception as well as in anxiety states. The anti-nociceptive system can also be activated by a variety of stressful stimuli such as footshock and social defeat (Grisel et al., 1993) and stimulation of the periaqueductal gray (PAG) in rat (Fardin et al., 1984). Since mice receive electric foot shock, it is possible that an analgesic action could account for the effects observed in the FPT. However, antidepressants (ADs) with different mechanisms of action, which are known to have analgesic properties (Yokogawa et al., 2002) are not active in the FPT (i.e., fluoxetine, imipramine) (Hascoët et al., 2000). Furthermore, the antidepressants (tricyclics, SSRIs, SNRIs), which possess analgesic properties, are active in the treatment of chronic pain in human (Sindrup and Jensen, 1999) but not in acute pain. A direct activation of the endogenous opioid system or potentiation of an analgesic effect mediated by serotoninergic and/or noradrenergic pathways or combinations of both are thought to be involved in their anti-nociceptive effect (Duman et al., 2004). Debate exists as

Abbreviations: AD(s), antidepressant(s); BZD(s), benzodiazepine(s); FPT, four-plate test; GABA, gamma-aminobutyric acid; 5-HT, 5-hydroxytryptamine, serotonin; NA, noradrenaline; PAG, periaqueductal gray; SSRI(s), selective serotonin reuptake inhibitor(s); SNRI, serotonin and noradrenaline reuptake inhibitor(s).

* Corresponding author. Tel.: +33 2 40412852; fax: +33 2 40412856.
E-mail address: michel.bourin@univ-nantes.fr (M. Bourin).

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to the nature of antidepressant-induced anti-nociception (Gray et al., 1998). In animal experiments and clinical studies, administration of ADs has yielded confusing results reporting anti-nociceptive or no effects (Otsuka et al., 2001). Studies have revealed the presence of at least four types of 5-HT receptors in the spinal cord (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4) (Millan, 2002). However, the exact nature of receptors involved in the 5-HT modulation of pain in the spinal cord remains to be elucidated (Bardin et al., 2000) and controversies exist concerning the possible role played by these receptors (Xu et al., 1994). For example, activation of the 5-HT_2 receptors has been reported both to facilitate and to inhibit the transmission of the nociceptive impulse (Eide and Hole, 1991).

Several lines of evidence indicate interactions between the opioid and GABAergic systems and suggest that acute activation of supraspinal $GABA_A$ receptors antagonises morphine-induced analgesia (Rady and Fujimoto, 1993). The acute administration of BZDs increased the pain threshold in rats (Wuster et al., 1980). Evidence exists that the interplay of BZDs and morphine on anti-nociception depends upon the BZD ligand, dose, mode of administration (acute or repeated) (Wala et al., 2001) and tests used (Rosland et al., 1990).

The hot-plate test is a behavioural model of nociception where organized behaviours such as hind paw-licking and jumping are elicited following noxious thermal stimulus. These reactions are controlled by supraspinal mechanisms. Licking is a rapid response elicited by painful thermal stimuli that is a direct indicator of nociceptive threshold (Espejo and Mir, 1993). In contrast, jumping represents a more elaborated response with a longer latency and encompasses an emotional component of escaping (Espejo et al., 1994). Some studies suggest that analgesia and the behavioural manifestations have different neurochemical substrates or that neuronal systems such as PAG in the rat may be independently triggered during the aversive state and the analgesia (Borges et al., 1988). Unpublished results in our laboratory have previously shown that at anxiolytic doses in the FPT, BZDs and ADs do not modify pain sensitivity in the hot-plate test.

The present study was thus designed to compare drug effects in the FPT and in the hot-plate test, in order to disambiguate drug-induced anti-punishment effects obtained in the FPT from alterations in pain sensitivity and to confirm our previous unpublished data. We have thus studied various compounds in the hot-plate test at anxiolytic and/or anxiogenic and/or inactive doses in the FPT: BZDs, such as diazepam and alprazolam and ADs with different mechanisms of action [SNRIs (imipramine, milnacipran and venlafaxine), selective noradrenaline reuptake inhibitors (desipramine and maprotiline), 5-HT_{2A} antagonist/serotonin reuptake inhibitor (trazodone), SSRIs (paroxetine and fluoxetine) and the dopamine reuptake inhibitor (nomifensine)]. The hot-plate results are compared with results previously obtained in the FPT (for ADs) (Hascoët et al., 2000). Furthermore, the effects of morphine and nomifensine in the FPT were compared with results in the hot-plate test. The locomotor activity, previously (Bourin et al., 1992, 1996, 2005; David et al., 2003; Redrobe et al., 1998) or not determined, was also discussed.

2. Methods

2.1. Animals

Male mice (Swiss strain) (Centre d'élevage Janvier, France) weighing 20–24 g were used throughout this study. They were housed in groups of 18 per cage (40 cm × 28 cm × 17 cm) on 12:12 light/dark cycle (light on 07:00 h) and had free access to food and water. The ambient temperature of the room was maintained at 21 ± 1 °C and the humidity was 50%. Experimental groups were composed of 8 to 12 mice. All experiments were performed according to the guidelines of the French Ministry of Agriculture for experiments with laboratory animals (law no. 87 848). Testing was performed between 09:00 and 13:00 h.

2.2. Drugs

Diazepam (RBI, Sigma, France), alprazolam (RBI, Sigma, France), paroxetine HCl (Smithkline Beecham, France), venlafaxine (Wyeth, France), milnacipran (Pierre Fabre, France), imipramine HCl (RBI, Sigma, France), desipramine (RBI, Sigma, France), fluoxetine (Lilly, France), maprotiline (RBI, Sigma, France), trazodone (UPSA), nomifensine (RBI, Sigma, France) and morphine (MO) HCl (Coopération Pharmaceutique Française, France) were used.

Paroxetine, venlafaxine and morphine were dissolved in distilled water and all other compounds were dissolved in a 5% concentration of Tween-80. All drugs or vehicle were administered intraperitoneally (i.p.) 30 min before the test in a volume of 0.5 ml/20 g of body weight.

2.3. Locomotor activity test (Boissier and Simon, 1965)

The spontaneous activity of naive animals was recorded using a photoelectric actimeter (OSYS). This apparatus consists of a transparent cage from which the animal's activity is measured by light beams connected to a photoelectric cell. The total number of horizontal cage crossings was recorded over a period of 10 min. The actimeter test was performed independently of the FPT in order to examine the effect of drugs on spontaneous locomotor activity of mice.

2.4. The hot-plate test (Jacob et al., 1974)

This test evaluates the analgesic potential of molecules (selective suppression of pain). The animal is placed on the metal plate heated to 55 °C surrounded by a glass cylinder (13 × 17 cm). The latency (in seconds) of the first jumping is measured. A cut-off time of 2 min is applied. Morphine (4 mg/kg) is used as an internal standard.

2.5. The FPT (Aron et al., 1971) (BIOSEB, France)

This apparatus consists of a cage (18 × 25 × 16 cm) floored by four identical rectangular metal plates (8 × 11 cm) separated from one another by a gap of 4 mm. The plates are connected to a device that can generate electric shocks (0.6 mA, 0.5 s). The top